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Seasonal growth potential of rare lake water bacteria suggest their disproportional contribution to carbon fluxes

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Summary

We studied the seasonal growth potential of opportunistic bacterial populations in Lake Zurich (Switzerland) by a series of grazer free dilution culture assays. Pronounced shifts in the composition of the bacterial assemblages were observed within one doubling of total cell numbers, from initially abundant *Actinobacteria* to other fast growing microbial lineages. Small populations with growth potentials far above community average were detected throughout the year with striking seasonal differences in their respective taxonomic affiliations. Members of *Cytophaga-Flavobacteria* (CF) were disproportionally proliferating only during phytoplankton blooms in spring and summer, while *Beta*- and *Gammaproteobacteria* showed superior growth at all other occasions. Growth rates of *Alphaproteobacteria* and esp. *Sphingomonadaceae* were significantly correlated to water temperatures and were far above community average in summer. Within the genus *Flavobacterium,* two species-like populations showed a tendency for fast growth in most experiments, while four others were exclusively proliferating either during a spring or during a summer phytoplankton bloom. Their high growth potentials but low *in situ* abundances hint at a tight control by bacterivorous grazers, and at a consequently accelerated carbon flux to higher trophic levels.

Introduction

Nutrient and substrate availability are crucial factors for the growth of osmotrophic aquatic microorganisms (Pernthaler and Amann, 2005; Thingstad et al., 2005; Šimek et al., 2006). Pelagic habitats are far from being static; the light-penetrated upper water strata of lakes are particularly dynamic, as substrate quantity and composition (of e.g., amino acids or sugars) undergo pronounced seasonal and diel changes and may fluctuate at even shorter time scales (Meon and Jüttner, 1999; Weiss and Simon, 1999). A significant fraction of the organic carbon readily available for bacteria is derived from algae (Hama and Handa, 1987; Giroldo et al., 2007; McCallister and del Giorgio, 2008). As phytoplankton undergoes strong temporal changes driven by physico-chemical factors and grazing by zooplankton (Sommer et al., 2012), bacteria depending on algal exudates might also be affected substantially, with diverging populations favoured at particular times (Eckert et al., 2012; Teeling et al., 2012).

Pelagic microbial assemblages show high physiological and ecological diversity, as they do not only differ in taxonomic composition, but also in terms of morphology, cell size, growth strategy, or substrate preference (Hahn, 2006; Šimek et al., 2006; Posch et al., 2009; Salcher et al., 2013). Some members of the bacterioplankton, so-called opportunistic or copiotrophic microbes, can quickly react to sudden nutrient and substrate pulses and reach growth rates far above community average (Beardsley et al., 2003; Šimek et al., 2005; Lauro et al., 2009). In terms of life style, they might be placed between permanently free-living taxa such as LD12 *Alphaproteobacteria* (Salcher et al., 2011b) and ac1 *Actinobacteria* (Allgaier and Grossart, 2006) and other, predominantly particle-attached genotypes (Parveen et al., 2011). Although the abundance of microbes with an opportunistic growth strategy is usually low in the pelagic zone (Šimek et al., 2006; Nelson and Carlson, 2012), their contribution to bacterial biomass production and carbon fluxes to higher trophic levels can nevertheless be substantial (Šimek et al., 2005; Posch et al., 2009; Zeder et al., 2009). At a first glance it may seem paradoxical that these prokaryotes are rare and total bacterioplankton abundances are

rather stable throughout the season (Pernthaler and Posch, 2009; Pedrós-Alió, 2012). However, selective mortality may limit the dominance of the fastest growing bacteria. Predation by phagotrophic protists and viral lysis are believed to affect most strongly the large and fast growing bacteria (Pace and Cole, 1994; Pernthaler, 2005; Rodriguez-Brito et al., 2010). As opportunistically growing bacteria typically show larger than average cell sizes, they are highly vulnerable to size selective grazing (Beardsley et al., 2003; Šimek et al., 2013).

Examples for such a life strategy can be found among freshwater *Betaproteobacteria* of the genus *Limnohabitans* (Šimek et al., 2005) and copiotrophic marine *Gammaproteobacteria* (e.g., *Alteromonas* sp., *Vibrio* sp., and *Pseudoalteromonas* sp.) (Beardsley et al., 2003; Nelson and Carlson, 2012). Genera of the latter group may even conserve high numbers of ribosomes during periods of starvation to quickly react on sudden nutrient pulses (Eilers et al., 2000; Beardsley et al., 2003). The *Flavobacteria* are another highly diversified phylogenetic group of opportunistically growing microbes in pelagic habitats that can form numerous small and transient populations in freshwater plankton (Eiler and Bertilsson, 2007; Zeder et al., 2009; Teeling et al., 2012). *Flavobacteria* are often favoured by phytoplankton bloom derived exudates or decay products (Newton et al., 2011). They can be enriched on algal exudates (Nelson and Carlson, 2012; Sarmento and Gasol, 2012), cultivated on solid media (Cousin et al., 2008), and are typically abundant during phytoplankton blooms (Salcher et al., 2010; Eckert et al., 2012; Teeling et al., 2012).

In this study, we aimed to analyse taxonomic affiliations and seasonal variations in the growth potential of opportunistic bacteria in Lake Zurich (Switzerland). We hypothesized (i) that there would be a high diversity of bacteria with an opportunistic life strategy; (ii) that their growth potentials would change with the season; and (iii) that *Flavobacteria* would proliferate particularly well during phytoplankton blooms. These hypotheses were tested in a series of short term dilution cultures with elevated nutrient and substrate availability per

bacterium (via 1:10 dilution with sterile lake water) and reduced grazing (via filtration). This simultaneous decoupling from top-down and bottom-up control allowed for an enrichment of opportunistically growing bacteria at close to native conditions, i.e., without changing ambient substrate and nutrient quality (Zeder et al., 2009). Six experiments were conducted at different time points to assess the growth potential of bacterial populations during the limnological year (i.e., winter, onset and decline of phytoplankton spring bloom, clear-water phase, decline of phytoplankton summer bloom, and autumnal mixis).

Results

Limnological background and timing of the experiments

Lake Zurich was thermally stratified between April and December (Fig. 1). At the beginning of the year, the filamentous cyanobacterium *Planktothrix rubescens* was the dominant primary producer at 5 m depth (Fig. 1). With the establishment of thermal stratification, *P. rubescens* formed a stable layer at the lower boundary of the metalimnion (9-15 m depth, data not shown), and was no longer detectable in the epilimnion between May and late August. A spring phytoplankton bloom occurred in April/May with a rapid development of cryptophytes, chlorophytes, and diatoms, which dominated in later phases of the bloom. Chlorophyll *a* concentrations quickly decreased during the clear-water phase in May. The following summer phytoplankton bloom was initially dominated by chlorophytes followed by high abundances of diatoms. With the beginning of surface mixis in September, *P. rubescens* got entrained to the epilimnion, finally dominating the phytoplankton community from October on.

Dilution culture experiments were performed during periods dominated by *P. rubescens* (winter: Feb 02), by *P. rubescens* and diatoms (onset of spring bloom: Apr 03, and autumn: Sep 28*),* by diatoms (decline of spring bloom: Apr 23, and decline of summer bloom: Jul 27), and during the clear-water phase (May 27) when chlorophyll *a* concentrations reached their

113 seasonal minimum (Fig. 1). Additional experiments were conducted in summer 2013 (Aug
114 06) to estimate the impact of grazing by flagellates and reduced bottom-up limitation (Fig. 7).
115
116 *Abundances and activity status of microbes in 1:10 dilution cultures*
117 Total microbial cell numbers ranged from 1.3 - 3.3 x 10^5 cells ml^{-1} at the beginning of
118 the dilution cultures (Fig. 2) and doubled within 36 - 196 h (Table 1). Growth rates, calculated
119 based on doubling times, were significantly correlated to *in situ* water temperatures (Pearson's
120 correlation coefficient $r = 0.69$, $p < 0.0001$). HNA bacteria increased on average by 3.6 fold
121 during the experiments (Fig. S1), pointing to the growth of highly active microbes. Short
122 termed incubations with radiolabeled glucose and leucine at the beginning and end of the
123 dilution cultures showed a different trend (Fig. 2): A significant increase in the fraction of
124 cells incorporating both tracers was only detected at the onset of the phytoplankton spring
125 bloom (Apr 03), while in most other cases the proportion of cells with uptake of either
126 glucose or leucine decreased in the dilution cultures.
127
128 *Changes in the taxonomic composition of bacteria*
129 *Actinobacteria* were the most abundant microbes in the original water samples, with
130 maximal proportions of 28% and 24% of DAPI stained bacteria in late spring and autumn
131 (mean 18.3%, Fig. 3). While their contribution to the total assemblage drastically decreased
132 during dilution cultivation (mean 8.2%), their total cell numbers remained stable, except for
133 winter (Feb 02, significant decrease by 50%, Fig. 3). *Betaproteobacteria* constituted more
134 than 10% of bacterioplankton in most of the original samples. They were particularly
135 successful in the dilution cultures from the onset of the phytoplankton spring bloom (Apr 03),
136 where they reached relative abundances of 49%, but not in experiments at the decline of
137 phytoplankton blooms (Apr 27, Sep 28, negative correlation of *Betaproteobacteria* to *in situ*
138 chlorophyll *a* concentrations: $r = -0.82$, $p < 0.001$). The largest part of *Betaproteobacteria*

was affiliated with the ubiquitous *Limnohabitans* spp. tribe targeted by probe R-BT065 (16 - 45% and 25 - 87% of *Betaproteobacteria* at the beginning and end of the experiments, respectively, Table S1). *Cytophaga-Flavobacteria* (CF) as targeted by FISH probe CF319a showed relative abundances between 3.7 - 9.4% in the original samples. These bacteria dominated in dilution cultures performed at the decline of both phytoplankton blooms (Apr 23 and Jul 27) in terms of growth rates, enrichment factors, and relative abundances at the end of the experiments (Fig. 3). Their growth in the six dilution cultures was significantly correlated to the *in situ* concentrations of diatom-derived chlorophyll *a* at the respective time points (r = 0.63, p = 0.003). *Alphaproteobacteria* were generally less abundant in the original samples with maximal percentages of 5.6%. Their growth rates in dilution cultures were among the highest at the decline of the summer phytoplankton bloom (Jul 27), and they were significantly correlated to *in situ* water temperatures (r = 0.82, $p < 0.001$). The majority of *Alphaproteobacteria* could be attributed to *Sphingomonadales* targeted by a newly designed probe (Table 2, Table S1). Their growth rates and abundances were highest during summer (17% of DAPI) and were significantly correlated to all *Alphaproteobacteria* (r = 0.93, $p <$ 0.001) and water temperatures (r = 0.87, $p < 0.001$). *Gammaproteobacteria* were clearly the least abundant bacteria in the original samples, however, they dominated the assemblage in 2 experiments (winter: Feb 02, and clear-water phase: May 27) and had high growth rates and enrichment factors in all experiments except for the phytoplankton bloom situations (negative correlation between chlorophyll a and growth rate: r = -0.78, p = 0.001).

Growth potential of different flavobacterial subpopulations

A total of 360 full and partial 16S rDNA sequences were obtained from two clone libraries gained from samples taken at the end of the experiments at the decline of phytoplankton blooms in spring (Apr 23, 129 sequences) and in summer (Jul 27, 231 sequences). *Flavobacteria* sequences were the most abundant in both clone libraries (96 in

spring, 75 in summer) and were distributed among 9 species-like monophyletic clusters (within-cluster sequence identities: 98.6 - 99.3%, Fig. 4). All these clusters were composed of sequences exclusively derived either from the spring or from the summer experiments, i.e., 3 clusters were exclusive for spring and 7 clusters were exclusive for summer. Sixty-one nearly complete 16S rDNA sequences representing those clusters were used for probe design. Three of these probes targeted populations with very low abundances that were not analysed further (data not shown).

Six novel species-like lineages of *Flavobacteria* were quantified by CARD-FISH (Table 2). Between zero and four of these populations were detectable at any single time point (Fig. 5). Together they constituted up to 4% (mean: 1.4%) of DAPI stained cells in the lake water samples and up to 56% (mean: 22%) in dilution cultures (Table S1). *Flavobacteria* targeted by probe Flav2-438 and by the newly designed probe FlavF-185 were growing in all dilution cultures except for autumn (Sep 28), where all flavobacterial lineages were below detection limit (< 0.14% of DAPI counts). Flav2 was the most abundant lineage *in situ* at all time points except for the spring phytoplankton bloom decline (Apr 23), where bacteria hybridized with probe FlavC-1266 were more abundant (Fig. 5). Flav2 constituted on average 19% of all *Flavobacteria* in the original samples and up to 84% at the end of the experiments (Table S1). FlavF were below the detection limits in all original samples (<0.14% of DAPI counts) but showed the highest growth rates and enrichment factors of *Flavobacteria* in all dilution cultures except for the decline of the summer phytoplankton bloom (Jul 27, Table S1). The remaining lineages of *Flavobacteria* were detectable at one particular time point either at the decline of the spring (FlavB and FlavC, Apr 23) or the summer phytoplankton bloom (FlavE and FlavCHE, Jul 27). Almost 90% of all CF were identified at the end of the experiments conducted during spring phytoplankton decline by applying 4 specific probes (Fig. 5, Table S1). FlavC reached relative abundances of 38% of all bacteria (60% of CF), and FlavB was present in 10% of DAPI, while FlavF and Flav2 played only a minor role (<5% of DAPI).

191 The experiments at the decline of the summer phytoplankton bloom were dominated by
192 FlavCHE with growth rates of 3.3 d^{-1} and relative abundances of one quarter of all bacteria
193 (54% of CF).
194
195 *Effect of incubation temperature, dilution, and grazing by flagellates*
196 Temperature induced effects on growth and taxonomic composition of bacteria were
197 tested during summer (Jul 27, Table S1). Besides incubation at *in situ* water temperature
198 (21°C), additional dilution cultures were incubated at 8°C, representing the water temperature
199 in spring. This treatment resulted in a decrease in bacterial growth rates by a factor of >5, i.e.,
200 the first doubling of total cell numbers took place after 168 h at 8°C compared to 36 h at *in*
201 *situ* temperature. The two sets of dilution cultures showed no significant difference at a
202 taxonomic level of phyla to orders (HGC, BET, CF, and ALF) at the end of the experiments;
203 however, the growth success of species- to genus like bacterial lineages was clearly
204 influenced by temperature. Specifically, *Limnohabitans* spp. (*Betaproteobacteria)* reached
205 significantly higher relative abundances at 8°C than at *in situ* temperatures (Fig. 6, Table S1).
206 Three out of 4 flavobacterial lineages showed the same trend with 1.5-3 times higher relative
207 abundances at the end of the experiments in the 8°C treatments. By contrast, the dominant
208 *Flavobacteria* in the summer experiment, FlavCHE, were slightly negatively affected by
209 artificially decreased temperature, as well as *Sphingomonadales* (*Alphaproteobacteria*),
210 which were only half as abundant in these treatments.
211 Bacterial numbers significantly increased in additional grazer-free dilution cultures
212 conducted in summer 2013, but stayed relatively stable in dilution cultures containing
213 bacterivorous protists (Fig. 7). A slight increase in bacterial numbers was detected in
214 undiluted treatments without grazers, while abundances significantly decreased in untreated
215 water samples together with a decline in HNF numbers. The numerical ratio of bacteria to
216 flagellates was constant in the latter variant (862±37) as well as in the dilution cultures

217 containing protists (1096±43). No significant changes in the taxonomic composition of
218 bacteria (% of DAPI, data not shown) were observed in the untreated incubations, i.e., all
219 bacterial populations were declining to the same extent. Dilution cultures were dominated by
220 *Alpha*- and *Betaproteobacteria* in both variants (i.e., prefiltered and unfiltered) at the end of
221 the experiments. *Gammaproteobacteria* were always below detection limit and
222 *Actinobacteria* and CF stayed relatively stable in all treatments.
223
224 **Discussion**
225 *Competitive disadvantages of abundant oligotrophic bacteria*
226 The composition of the microbial assemblages in the original samples (Fig. 3) reflected
227 well the general seasonal patterns in temperate freshwater lakes, e.g., high proportions of
228 *Actinobacteria* in spring and/or autumn (Allgaier and Grossart, 2006; Salcher et al., 2010).
229 These microbes seem to be better protected from bacterivorous flagellates due to their minute
230 cell sizes and gram positive cell walls (Pernthaler et al., 2001; Jezbera et al., 2006; Tarao et
231 al., 2009), and they profit from organic carbon sources released by the consumption of their
232 grazing-vulnerable competitors (Eckert et al., 2013). However, *Actinobacteria* never reached
233 high densities in our predator-free dilution cultures (Fig. 3), hinting at competitive
234 disadvantages to opportunistic bacterial taxa (Burkert et al., 2003). Also microbes affiliated
235 with the ubiquitous alphaproteobacterial tribe LD12 were outcompeted in our dilution assays
236 (data not shown), confirming an oligotrophic lifestyle (Salcher et al., 2011b).
237
238 *Proliferation of opportunistic planktonic bacteria*
239 The simultaneous release from predation and substrate limitation induced rapid growth
240 and consistently favoured bacteria with high nucleic acid content (HNA) (Fig. 2). While this
241 could indicate a general activation of microbes (Lebaron et al., 2001; Salcher et al., 2007), the
242 uptake of radiolabeled glucose and leucine seemed to suggest otherwise: the proportions of

243 cells incorporating the offered compounds typically decreased towards the end of the
244 experiments, except for one experiment conducted at the onset of the phytoplankton spring
245 bloom (April 03, Fig. 2). This seemingly paradox was most likely related to the specific
246 substrate preferences of the enriched bacteria: microbes targeted by probe R-BT065 and other
247 *Betaproteobacteria* (dominant on April 03) were highly involved in glucose and leucine
248 uptake, whereas almost no CF (dominant at other instances) were found to incorporate either
249 tracer (data not shown). Such population specific uptake of low molecular weight compounds
250 is in line with previous findings in Lake Zurich (Eckert et al., 2012; Salcher et al., 2013). Our
251 results from tracer incorporation might, therefore, reflect the general changes in community
252 composition rather than the actual bacterial activities.
253
254 *Temperature dependent growth of opportunistic microbial populations.*
255 Generally, total bacterial growth rates were positively correlated with water temperature
256 ($r = 0.69$, $p < 0.0001$), as often observed *in situ* (White et al., 1991; Coveney and Wetzel,
257 1995), and *in vitro* (Carlsson and Caron, 2001). We compared the effect of artificially lowered
258 temperature (8°C vs. 21°C) in summer (Jul 27, Fig. 6). Although growth rates were much
259 lower at 8°C, no significant differences of the bacterial assemblages on a phylum to class
260 level were detectable. However, significant differences were found at a higher phylogenetic
261 resolution, as two out of five species- to genus-like populations were more abundant at 8°C
262 than at 21°C (Fig. 6). These populations (*Limnohabitans* spp. and FlavF) reached highest
263 densities in dilution cultures in winter and spring, when water temperatures were below 8°C
264 indicating that these microbes might be cold-stenotherm. *Sphingomonadales* on the other
265 hand were 2 times more abundant in treatments incubated at *in situ* temperature (21°C),
266 pointing to warm-stenothermic growth, as previously suggested in experiments with elevated
267 water temperatures (Dziallas and Grossart, 2011).
268

Growth of opportunistic bacteria and quality of dissolved organic matter (DOM).

Our dilution approach selected for the fastest growing microbes at close to native conditions, i.e., without changing ambient substrate and nutrient quality (Jüttner et al., 1997; Meon and Jüttner, 1999), thus, we specifically enriched bacteria with an opportunistic lifestyle. These 'growth specialists' were affiliated with *Proteobacteria* and CF, however, growth rates of these taxonomic groups varied drastically between different experiments (Fig. 3). This might be linked to seasonal variations of the chemical composition of DOM. In the epilimnion of Lake Zurich, DOM is predominantly allochthonous or recalcitrant for most of the year, and dissolved organic carbon (DOC) reaches annual minimal concentrations in winter (Salcher et al., 2011b). Maximal DOC concentrations were determined during phytoplankton blooms in spring and summer when production of autochthonous DOM is high (Table 1) (Zeder et al., 2009; Eckert et al., 2012). Most planktonic bacteria preferentially utilize autochthonous carbon sources (Kritzberg et al., 2004) and enrichment with autochthonous or allochthonous DOM differently affect the composition of bacterial assemblages (Pérez and Sommaruga, 2006; Nelson and Carlson, 2011). During summer stratification, the epilimnion of Lake Zurich is usually phosphate depleted, while early spring water turnover transports phosphorus rich hypolimnetic water to the surface (Table 1) (Posch et al., 2012). Therefore, bacteria in Lake Zurich might be limited by carbon in winter (until the spring bloom) and by phosphorus during summer, as observed for other lakes (Simon et al., 1998; Carlsson and Caron, 2001). Additionally, low ammonium concentrations might be limiting throughout the whole year, except for the clear-water phase between May and June (>30 µg NH_4-N l^{-1}, Table 1). However, bacterial nitrogen demands might also be covered by other sources such as dissolved free amino acids.

Ecological properties of opportunistic bacterial taxa

294 The majority of fast growing *Betaproteobacteria* were affiliated with the ubiquitous
295 *Limnohabitans* tribe targeted by probe R-BT065 (Šimek et al., 2001; Newton et al., 2011),
296 whereas no other typical freshwater betaproteobacterial populations, such as
297 *Polynucleobacter* sp., or microbes from the LD28, bet I-B, bet I-C, bet III, and bet VI tribes
298 (Wu and Hahn, 2006; Salcher et al., 2011a; Salcher et al., 2013) profited from the dilution
299 assays (data not shown). However, *Limnohabitans* spp. proliferated only at times of low
300 primary production (Table S1) with growth rates far below reported maxima of 1.6 d^{-1} (Šimek
301 et al., 2006). *Limnohabitans* spp. quickly respond to nutrient amendments, esp. phosphorus
302 (Šimek et al., 2003; Pérez and Sommaruga, 2006; Peura et al., 2012) and are versatile in their
303 incorporation of low molecular weight substrates (Salcher et al., 2013). In our study,
304 *Limnohabitans* spp. reached highest numbers at elevated phosphorus concentrations (i.e.,
305 winter and onset of phytoplankton spring bloom, Feb 02 and Apr 03, Table S1). The low
306 growth potential of *Limnohabitans* spp. during algal blooms seems somewhat in contrast to
307 previous observations (Salcher et al., 2008; Šimek et al., 2008; Eckert et al., 2012), i.e., a
308 close coupling to phytoplankton derived DOC (Pérez and Sommaruga, 2006; Šimek et al.,
309 2011). However, individual taxa of the genus *Limnohabitans* differ in growth behaviour and
310 substrate acquisition (Kasalický et al., 2013), and there are species-specific responses to
311 different algae, such as enhanced growth in the presence of cryptophytes and no growth with
312 desmidiaceae and cyanobacteria (Horňák et al., 2008; Šimek et al., 2011).
313 Highest growth rates of *Gammaproteobacteria*, matching previously reported maxima
314 of 1.35 d^{-1} (Šimek et al., 2006), were observed during the clear-water phase (May 27) at
315 elevated ammonium concentrations ($r = 0.56$, $p = 0.008$, Fig. 3), and they were also
316 proliferating at all other experiments except for those conducted during phytoplankton
317 blooms. Pelagic *Gammaproteobacteria* can quickly dominate microbial assemblages after
318 nutrient additions and / or removal of grazers (Eilers et al., 2000). They were enriched in
319 nutrient additions in autumn, but not during spring or summer in eutrophic Lake Mendota

(Newton and McMahon, 2011), and only at times of lowest phytoplankton biomass in a boreal lake (Peura et al., 2012). *Aeromonas hydrophila (Gammaproteobacteria),* known for its chitinolytic activity (Jagmann et al., 2010), was readily enriched in freshwater microcosms amended with algal-derived particles, but lost in direct competition assays against a strain affiliated with CF (Styp von Rekowski et al., 2008). Thus, there are indications that the growth of opportunistic pelagic *Gammaproteobacteria* is induced by recycled or allochthonous DOM sources and, to a lesser extent, also by phytoplankton exudates, but that they might be outcompeted by other taxa during algal blooms.

Alphaproteobacteria and therein *Sphingomonadales* were growing to high numbers only in summer (Jul 27, Fig. 3) and their growth was significantly correlated with water temperature. Most alphaproteobacterial sequences were affiliated with *Sphingopyxis* sp. (19 out of 22, data not shown). *Sphingopyxis* relatives were found to be associated with the diatom *Stephanodiscus minutulus* (Eigemann et al., 2013), while other *Sphingomonadaceae* occurred during cyanobacterial blooms (Eiler and Bertilsson, 2004; Dziallas and Grossart, 2011). Some *Sphingomonadaceae* can degrade recalcitrant organic compounds such as humic substances or phenol (Hutalle-Schmelzer et al., 2010), and *Sphingopyxis* spp. numbers increased in grazer-free dialysis bags in the dystrophic compartment of an artificially divided lake (Grossart et al., 2008). However, even closely related *Sphingomonadaceae* showed distinct seasonal patterns and differed greatly in substrate uptake (Jogler et al., 2011), suggesting a high degree of specialization.

Algal derived DOM selects for different populations of Flavobacteria.

Although CF dominated in both experiments conducted at the decline of algal blooms (Fig. 1, Fig. 3), there were different 'bloom specialists', i.e., populations of *Flavobacteria* (Figs. 4, 5). Only two populations (Flav2 and FlavF) were detectable at both occasions; they formed one eight and one quarter of all *Flavobacteria* in spring and summer, respectively, and

346 dominated the opportunistic *Flavobacteria* during all other experiments except for autumn
347 (Sep 28, Fig. 5). Consequently, they might be regarded as being more generalistic. By
348 contrast, four other flavobacterial populations were exclusively proliferating either in spring
349 or in summer (Fig. 5), pointing to specific adaptations to DOM quality, e.g., to different types
350 of algal exudates. The algal blooms in Lake Zurich at both occasions consisted of mainly
351 diatoms and chlorophytes (Fig. 1), albeit of different genera: The diatoms *Stephanodiscus*
352 spp., *Tabellaria* spp., and *Diatoma* spp. were only present in spring, *Cyclotella* spp. and
353 *Asterionella* spp. in both blooms, and *Fragilaria* spp. was a typical summer phytoplankter.
354 Cryptophytes (esp. *Rhodomonas* sp.) and the dinoflagellates *Gymnodinium* sp. grew only in
355 spring, while *Peridinium* sp. was more abundant during summer. Other algae were present at
356 both occasions, but more abundant during summer, e.g., different genera of chlorophytes and
357 chrysophytes. Algal exudates, as well as bacterial-algal associations may be highly species-
358 specific (Giroldo and Vieira, 2005). Marine diatom and dinoflagellate blooms harboured
359 distinct CF populations depending on the physiological status of algal species (Grossart et al.,
360 2005; Mayali et al., 2011), and CF also dominated in experiments with augmentation of
361 marine phytoplankton (Simon et al., 2012). Transient, short-lived CF populations in the
362 coastal North Sea were specialized on algal derived high molecular weight compounds
363 (Teeling et al., 2012). Also in freshwaters, different populations of CF were abundant during
364 algal blooms (Eiler and Bertilsson, 2007; Eckert et al., 2012). Microbes affiliated with Flav2
365 dominated CF in grazer free dilution cultures from Lake Zurich shortly before and during the
366 spring phytoplankton bloom, but were less abundant at the decline of the spring bloom (Fig.
367 5) (Zeder et al., 2009), when *Flavobacteria* targeted by probes FlavB-992 and FlavC-1266
368 represented more than 75% of all CF at the end of the experiment (Apr 23, Fig. 5). Microbes
369 affiliated with the FlavC tribe are close relatives of *Flavobacterium aquatile,* as well as of the
370 'Faq' freshwater cluster that was enriched upon nutrient addition in a eutrophic lake during
371 spring, but not during summer or autumn (Newton and McMahon, 2011). An OTU closely

related to *F. aquatile* and *F. psychrophilum* increased in a nutrient amendment experiment conducted in an oligotrophic high mountain lake only at times of high chlorophyll *a* concentrations (Nelson and Carlson, 2011). This striking seasonality is in line with our observations. At the decline of the summer phytoplankton bloom almost 60 % of *Flavobacteria* were affiliated with the tribes FlavCHE and FlavE (Jul 27, Fig. 5). The probe FlavCHE-169 also targets *Flavobacterium cheniae*, a microbe isolated from the sediment of an eutrophic reservoir (Qu et al., 2008). This species does not assimilate carbohydrates, cellulose, pectin, and chitin, but is able to degrade gelatine (Qu et al., 2008). Notably, microbes affiliated with FlavCHE had the highest of all measured growth rates (Table S1), with doubling times of only 5.1 h, which by far exceeded published values for comparatively unproductive systems such as Lake Zurich (Coveney and Wetzel, 1995; Smith and Prairie, 2004).

Consequences for the carbon flux to higher trophic levels

We successfully enriched transient, opportunistic microbial populations in our dilution assays. Their high growth rates together with low *in situ* abundances hint at a tight control by bacterivorous grazers, and at a consequently accelerated carbon flux to higher trophic levels. This assumption was tested in a set of additional experiments (Fig. 7), where microbes were either facing (i) top-down and bottom-up limitation (untreated incubations), (ii) reduced top-down control (grazer-free incubations), (iii) reduced bottom-up limitation (1:10 dilution cultures), or (iv) reduced top-down and bottom-up limitation (grazer-free dilution cultures). Significantly less bacteria were present in treatments with grazers (i.e., -33% corresponding to 1.3×10^5 cells ml^{-1} and -42% corresponding to 1.6×10^6 cells ml^{-1} in diluted and undiluted samples, respectively, Fig. 7). By assuming an ingestion rate of 20.3±4 bacteria $flagellate^{-1}$ h^{-1} (determined via uptake rates of fluorescently labeled bacteria in Lake Zurich, M.M. Salcher, unpublished), we could assign 82±16% and 79±15% of the observed differences to grazing

losses by flagellates in the dilution cultures and undiluted samples, respectively. Although ingestion rates of different bacterial populations were not quantified, *Alpha-* and *Betaproteobacteria* were observed in food vacuoles of flagellates (data not shown). An ingestion rate of 20.3 bacteria flagellate^{-1} h^{-1} is within the range of published data from other habitats (i.e., 2 – 56 bacteria flagellate^{-1} h^{-1}) (Šimek et al., 2004; Šimek et al., 2008; Šimek et al., in press). We cannot exclude viral lysis as an important bacterial mortality factor, neither the possibility of synergistic interactions between viruses and protistan grazers (Šimek et al., 2001). However, initial viral loads can be assumed to be equal in both variants of our diluted and undiluted set-ups and an artificial removal of grazers does not seem to influence virus induced bacterial mortality in early stages of incubations (Šimek et al., 2001). We kept incubation periods as short as possible to minimize any effects of viruses in our experiments (Table 1). Thus, the majority of observed differences in bacterial growth in variants with vs. without grazers could be attributed to protistan predation. In conclusion, top-down control by protists seems to be a major cause for low *in situ* abundances of opportunistic bacterial populations, resulting in an accelerated carbon flux to higher trophic levels.

Experimental procedures

Study site and sampling

Lake Zurich is an oligo-mesotrophic prealpine lake (65.06 km^2 surface area, 136 m maximal depth, 1811 km^2 catchment area) dominated by the filamentous cyanobacterium *Planktothrix rubescens* (Posch et al., 2012). Sampling was conducted at the deepest point (N47°18’8.82” E8°34’42.91”) between Jan - Dec 2009 in a biweekly manner. Depth profiles of total and group specific chlorophyll *a* were determined with a multi wavelength probe (TS-16–12 fluoroprobe, bbe Moldaenke GmbH, Kronshagen, Germany), that was calibrated to distinguish different phytoplankton groups according to their pigment fluorescence spectra (Beutler et al., 2002; Salcher et al., 2011a) while temperature was measured with a multi-

parameter probe (6600 multi-parameter, water quality monitoring, YSI incorp., Yellow Springs, OH, USA). Water samples for dilution cultures were taken from 5 m depth at 6 selected dates (i.e., Feb 02, Apr 03, Apr 23, May 27, Jul 27, and Sep 28), delivered to the lab within 45 minutes, and immediately processed. Additional control experiments were conducted in Aug 06 2013 (Table 1).

Grazer free dilution cultures

Lake water free from bacteria was produced by filtration of lake water through 30 µm stainless steel nets followed by 0.1 µm hollow fiber filtration (polysulfone capillary module SEM-D-PS-AN-D-8A, 0.46 m^2 total filter area, Hydac Filtertechnik). The filtrate (1350 ml) was transferred into glass bottles (2 l, Schott) and inoculated with 150 ml of 0.8 µm filtered (0.8 µm pore size polycarbonate filters, 47 mm diameter, Whatman) lake water. Triplicates of the dilution cultures were incubated at *in situ* temperatures (and additionally at 8°C at Sep 27) in the dark until the first doubling of total cell numbers was observed (Table 1). The effects of 1:10 dilution and the presence vs. absence of protistan grazers were assessed in a set of additional experiments conducted in summer 2013 (Aug 06) at times of very high water temperature (25°C) and seasonal maxima of bacteria and flagellates (4 x 10^6 bacteria and 4 x 10^3 flagellates ml^{-1}). Notably, no pronounced algal summer bloom took place in 2013, i.e., chlorophyll *a* values were very low at the sampling date (Table 1). Triplicated set-ups (1500 ml each) of (i) untreated water samples, (ii) grazer-free (i.e., 0.8 µm prefiltered) water samples, and (iii) 1:10 dilution cultures were incubated along with (iv) grazer-free (i.e., 0.8 µm prefiltered) dilution cultures. Samples were taken every 12 - 48 h and fixed with formaldehyde (2% final concentration). Negative controls (600 ml of the 0.1 µm filtrate free from bacteria in 1 l bottles, Schott), that were incubated in parallel to the cultures, showed no growth of bacteria until the end of the experiments.

All glass devices and filtration towers were thoroughly rinsed with hydrochloric acid (2 M) and Milli-Q water, and were autoclaved prior to use. In order to minimise release or sorption of compounds, membrane filters were pre-rinsed with 500 ml of autoclaved Milli-Q and the hollow fibre module was autoclaved and rinsed with autoclaved Milli-Q for at least 60 min. The first 200 ml (membrane filters) and 3 l (hollow fibre module) of the filtrates were discharged. No enrichment of dissolved organic carbon was detectable via measurements of samples with a TOC analyser (TOC-5000, Shimadzu, Kyoto, Japan) prior and after hollow fibre filtration, as hollow fibre filtration is very gentle and has no effect on the integrity of fragile organisms and the concentration of organic compounds (Jüttner et al., 1997).

Bacterial abundance and composition

For microscopic cell counting 5-10 ml of sub samples were collected on black membrane filters (0.22 µm pore size, 25 mm diameter, Osmonics) and stained with 4'6'-diamidino-2'-phenylindole (DAPI) in final concentrations of 3 to 7 µg ml^{-1} (Porter and Feig, 1980). Proportions of high nucleic acid (HNA) cells were determined with an inFlux V-GS flow cytometer (Cytopeia Inc.) equipped with a blue laser (Sapphire HP, 488 nm wavelength, Coherent Inc). All samples were stained with the DNA stains Syto 13 or Syto 9 (Invitrogen) for a minimum of 30 min in the dark. Scatter plots were evaluated with the software FlowJo 7.2.2 (Tree Star Inc.) and gates for HNA cells were set as exemplified in Fig. S1.

CARD-FISH was conducted as previously described (Sekar et al., 2003; Eckert et al., 2012) with a reduced achromopeptidase pretreatment (30 min) to avoid overdigestion of fast growing cells. The following probes were used: ALF968, BET42a, GAM42a, specific for *Alpha*-, *Beta*-, and *Gammaproteobacteria*, respectively, CF319a detecting mainly *Flavobacteriales* (Amann and Fuchs, 2008), R-BT065 specific for *Limnohabitans* spp. (Šimek et al., 2001), and Flav2-438 specific for a small population of *Flavobacteria* (Zeder et al., 2009). Newly designed probes used in this study are listed in Table 2. DAPI and CARD-

FISH preparations were evaluated by fully automated high-throughput microscopy (Zeder and Pernthaler, 2009). Nine randomly chosen filter sections from different experiments were chosen for an estimation of the level of detection by CARD-FISH, which was calculated as average hybridization rate with a NON-EUB probe plus three-fold standard deviation (i.e., 0.031 + (3 x 0.036)) (Amann and Fuchs, 2008). The detection limit was 0.14% of DAPI stained particles in our dilution assays. All hybridization rates below this limit were set to b.d. (below detection) or <0.14% and excluded from figures.

Microautoradiography

Tracer uptake experiments were conducted with the inocula and one replicate of the dilution cultures at the end of the experiments. Triplicate samples (inocula: 6 ml, dilution cultures: 10 ml) and duplicates of prefixed controls (formaldehyde, 2% final concentration) were amended with [^{3}H]-Leucine (62 Ci mmol^{-1} specific activity, Amersham) or [^{3}H] Glucose (39 Ci mmol^{-1} specific activity, Amersham) in final concentrations of 10 nM. The samples were incubated for 120 min at *in situ* temperature in the dark before fixation. After filtration (white polycarbonate filters, type GTTP, 0.2 µm pore size, 25 mm diameter, Millipore), microautoradiography was performed as described in Salcher et al. (2008).

Cloning of 16S rRNA genes, phylogenetic analyses, and probe design

Two clone libraries were constructed at the end of the experiments conducted in spring (April 23) and in summer (July 27). Cloning and sequencing of bacterial 16S rRNA genes was conducted as described in Eckert et al. (2012). Phylogenetic analysis was performed with the ARB software package and the SILVA SSU reference database release 108 (Ludwig et al., 2004). Sequences were aligned using the SINA web aligner and alignments were subsequently manually optimized. High quality sequences associated with *Flavobacteria* (>1300 nucleotides, pintail value >90) were transferred to a maximum parsimony tree

consisting of several thousand high quality *Flavobacteria* sequences. The tree was optimized with the appropriate ARB tools (ARB_parsimony, ARB_edit) and sets of 200 to 300 sequences were chosen for further analyses. Bootstrapped maximum likelihood trees were constructed on a dedicated web server (Stamatakis et al., 2005). Nodes with bootstrap supports <60% were collapsed into multifurcations. Oligonucleotides probes were designed in ARB with the SILVA SSU reference database releases 98 (FlavB and FlavC) and 104 (FlavE, FlavF, FlavCHE, and Sphingo-866) with the ARB tools probe_design and probe_check. Hybridisation efficiencies, mismatch stability and the effectiveness of competitor oligonucleotides were analysed with the web tool Mathfish (Yilmaz et al., 2011). Stringent hybridisation conditions for CARD-FISH were obtained by testing different formamide concentrations (10-70%). All 16S rDNA sequences have been deposited to GenBank with the accession numbers KC886742-KC887059.

Statistical analysis

Prior to statistical analyses, all data of relative abundances (percentages) were arcsine(square root(x))-transformed and cell numbers and chemical variables were log(x + 1)-transformed to obtain normal distribution. Significant changes between original and enriched samples were identified by one way ANOVA and Tukey post hoc tests. Paired T-tests were applied to compare different dilution cultures and Pearson's correlation analyses (two-tailed) were used to examine direct relationships between chemical and/or biological parameters and different bacterial populations. All analyses were done with the software SPSS 16.

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736
737

738 Figure legends:

739 Figure 1: Seasonality of water temperature (Temp, °C) and additive chlorophyll *a*
740 concentrations (Chl *a*, µg l^{-1}) of the cyanobacterium *Planktothrix rubescens*, diatoms,
741 chlorophytes, and cryptophytes in 5 m depth of Lake Zurich. Triangles mark dates
742 where dilution experiments were carried out. Labels at the top indicate different
743 seasonal events. SpB, spring phytoplankton bloom; CW, clear-water phase; SuB,
744 summer phytoplankton bloom.

745 Figure 2: Total bacterial abundances, proportions of cells with high nucleic acid content
746 (HNA), and proportions of DAPI-stained cells with active incorporation of glucose and
747 leucine at the beginning (original) and end (enriched) of the dilution experiments. Error
748 bars represent the standard error of triplicate set-ups. Grey shadings indicate
749 phytoplankton blooms. n.d., not determined.

750 Figure 3: Left panel: Proportions of *Actinobacteria* (HGC), *Betaproteobacteria* (BET),
751 *Cytophaga-Flavobacteria* (CF), *Alphaproteobacteria* (ALF), and
752 *Gammaproteobacteria* (GAM) at the beginning (original) and end (enriched) of the
753 dilution experiments. Right panel: Growth rates and enrichment factors of the analysed
754 bacterial populations. Error bars represent the standard error of triplicate set-ups. Grey
755 shadings indicate phytoplankton blooms. b.d., below detection limit.

756 Figure 4: Bootstrapped maximum likelyhood tree of 16S rDNA sequences affiliated with
757 *Flavobacteria*. Species-like tribes including sequences from the spring bloom (April 23)
758 and the summer bloom (July 27) experiment are depicted in grey and black,
759 respectively. The scale bar represents 1% estimated sequence divergence.

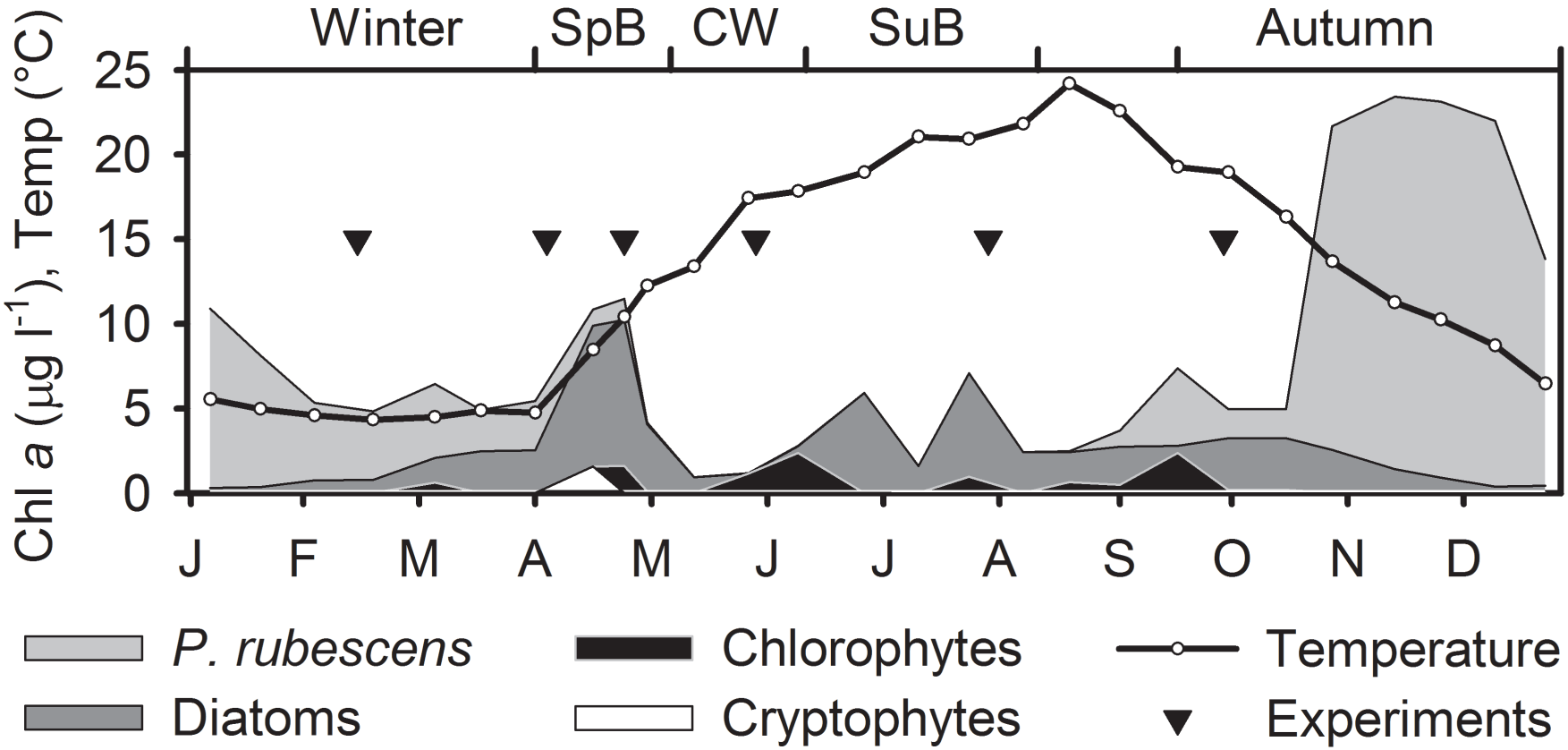
760 Figure 5: Proportion of different bacterial populations affiliated with *Flavobacteria* (CF) at
761 the beginning (upper panel, original) and end (lower panel, enriched) of the dilution
762 experiments. See Fig. 4 for phylogenetic positioning of different populations. SpB↑,

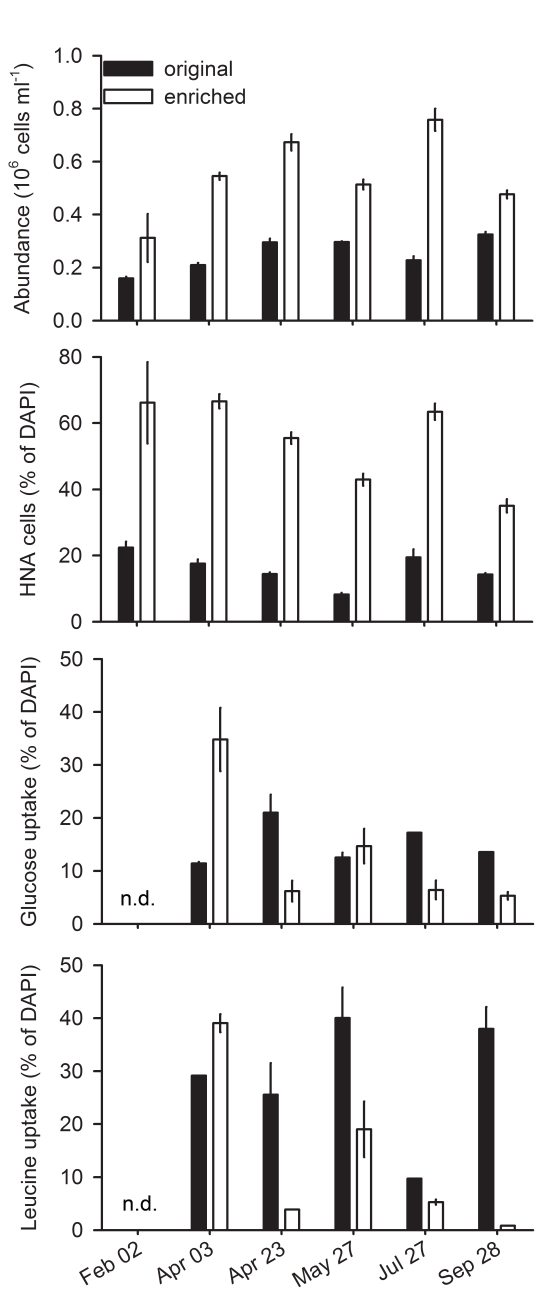
763 onset of spring phytoplankton bloom; SpB↓, decline of spring phytoplankton bloom;
764 CW, clear-water phase; SuB, decline of summer phytoplankton bloom.
765 Figure 6: Ratios of relative abundances of different bacterial populations at the end of dilution
766 experiments carried out at artificially lowered temperature (8°C) and at *in situ*
767 temperature (21°C) in July 27. Asterisks indicate significant differences between the
768 two treatments (** $p < 0.01$, * $p < 0.05$). Error bars represent the standard error of
769 triplicate set-ups.
770 Figure 7: Control experiments conducted in Aug 06 2013 with triplicated incubations of
771 grazer free dilution cultures, dilution cultures, grazer free lake water, and untreated lake
772 water. Top: relative changes in microbial abundances (bars: prokaryotes; circles:
773 flagellates). Bottom: Relative changes of bacteria associated with different bacterial
774 classes or phyla. Asterisks indicate significant changes during incubation (*** $p <$
775 0.001, ** $p < 0.01$, * $p < 0.05$). Different letters indicate significant differences
776 between treatments. Error bars represent standard errors of triplicate incubations. n.p.,
777 not present.

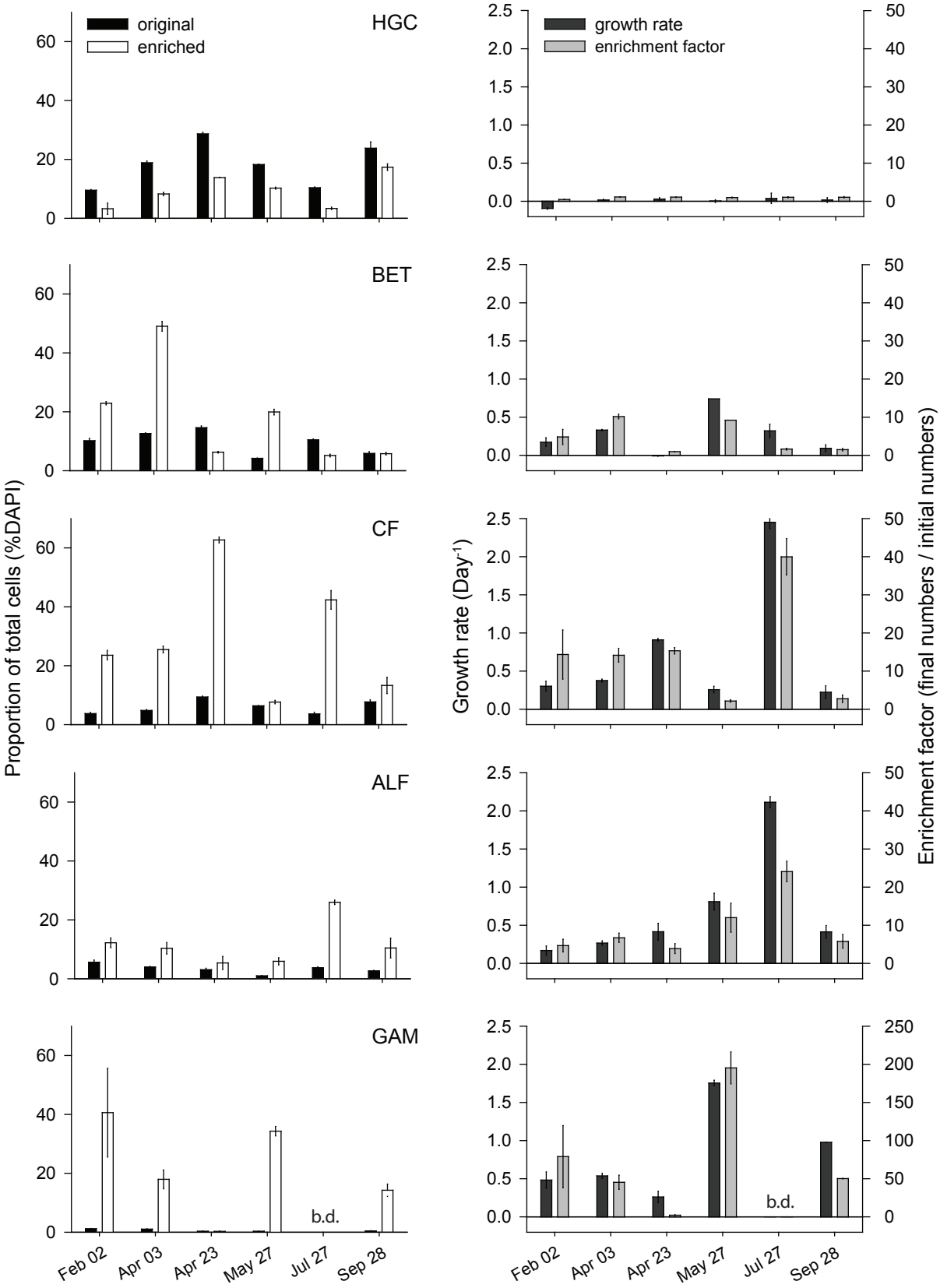
Table 1. Details of the different 1:10 dilution experiments. Experiments conducted during phytoplankton blooms are highlighted in grey.

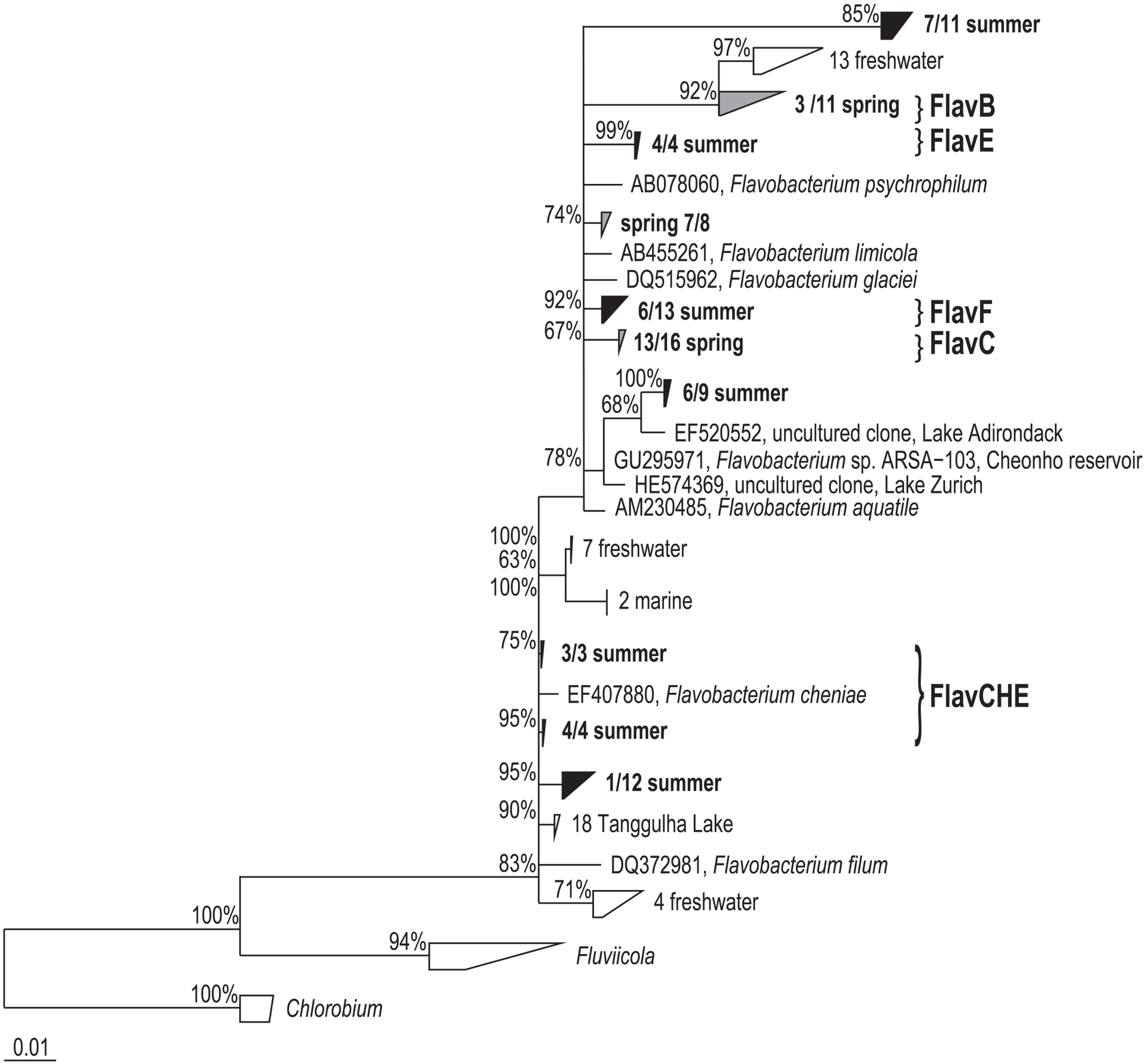
Date	Incubation period (h)	Relative growth	Temp (°C)	DOC (mg l^{-1})	DP (µg l^{-1})	NH_4-N (µg l^{-1})	Chl*a* tot (µg l^{-1})	Chl*a P.r* (µg l^{-1})	Chl*a* diat (µg l^{-1})	Chl*a* chlor (µg l^{-1})	Leu inc t_0 (pmol l^{-1} h^{-1})	Leu inc t_{end} (pmol l^{-1} h^{-1})	Glu inc t_0 (pmol l^{-1} h^{-1})	Glu inc t_{end} (pmol l^{-1} h^{-1})
Feb 04 2009	192	2.0 ± 0.6	4	1.41	6.8	4.1	2.68	2.35	0.26	0.06	n.d.	n.d.	n.d.	n.d.
Apr 03 2009	168	2.6 ± 0.1	5	1.67	7.1	1.3	2.84	1.23	1.61	0	51.2 ± 4.8	651 ± 48.1	54.7 ± 4.9	205.3 ± 9.8
Apr 23 2009	72	2.3 ± 0.1	9.8	1.86	1.4	5.6	9.72	1.05	7.32	1.36	215.8 ± 12.7	12.5 ± 1.0	155.3 ± 5.9	47.2 ± 3.6
May 27 2009	72	1.7± 0.0	14.1	1.41	0.4	30.8	1.75	0.03	1.01	0.71	163.2 ± 3.8	184.1 ± 9.4	191.4 ± 2.0	59.0 ± 2.0
Jul 27 2009	36	3.3 ± 0.1	21	1.41	2.7	n.d.	3.97	0	3.97	0	230.3 ± 23.2	71.7 ± 1.9	210.4 ± 11.9	136.3 ± 6.7
Jul 27 2009, 8°C	168	2.7 ± 0.1	8	1.41	2.7	n.d.	3.97	0	3.97	0	n.d.	n.d.	n.d.	n.d.
Sep 28 2009	96	1.5 ± 0.1	14	1.5	3.2	5.1	3.37	1.35	1.42	0.6	139.2 ± 1.6	39 ± 2.7	256.4 ± 4.3	11.2 ± 1.8
Aug 06 2013	14	1.5 ± 0.1	25.2	n.d.	n.d.	n.d.	2.50	0	1.87	0.63	n.d.	n.d.	n.d.	n.d.

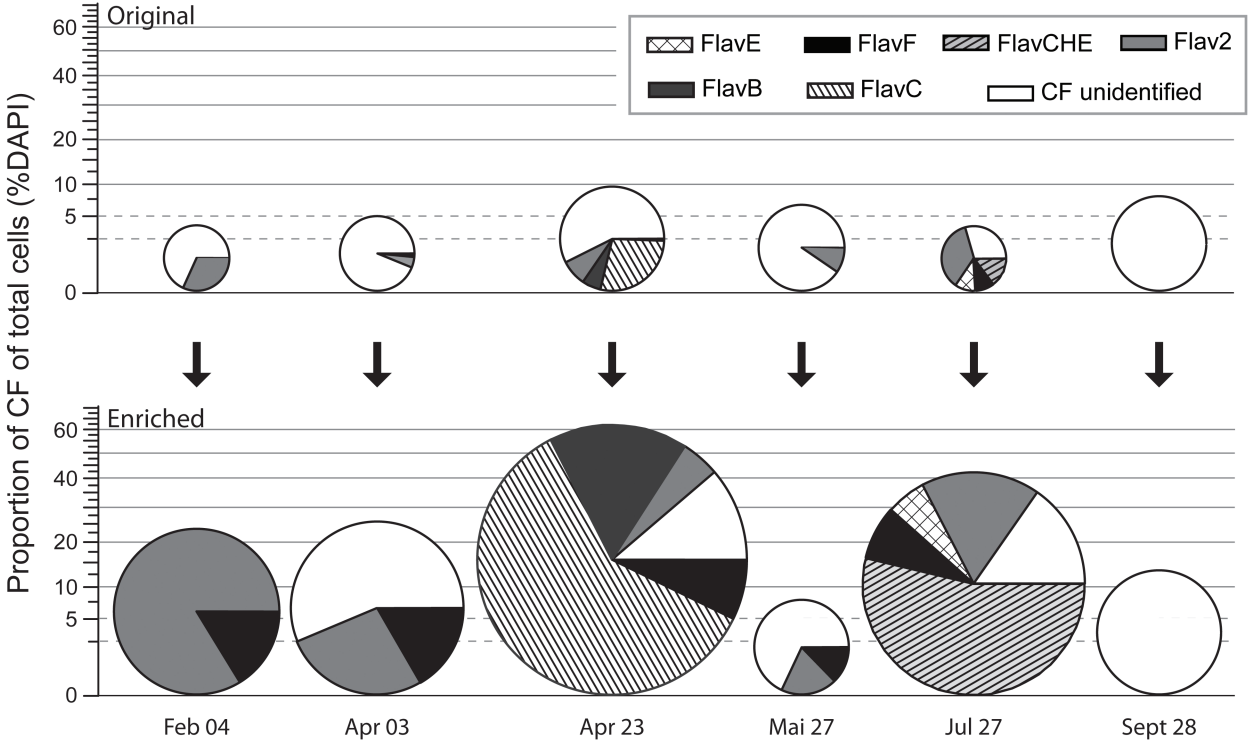
Temp, water temperature; DOC, dissolved organic carbon; DP, dissolved phosphorus; Chl*a* tot, total chlorophyll *a* concentrations; Chl*a P. r*, chlorophyll *a* concentrations assigned to *Planktothrix rubescens*; Chl*a* diat, chlorophyll *a* concentrations assigned to diatoms; Chl*a* chlor, chlorophyll *a* concentrations assigned to chlorophytes; Leu inc, leucine incorporation; Glu inc, glucose incorporation; t_0, beginning of experiments; t_{end}, end of experiments; n.d., not determined.

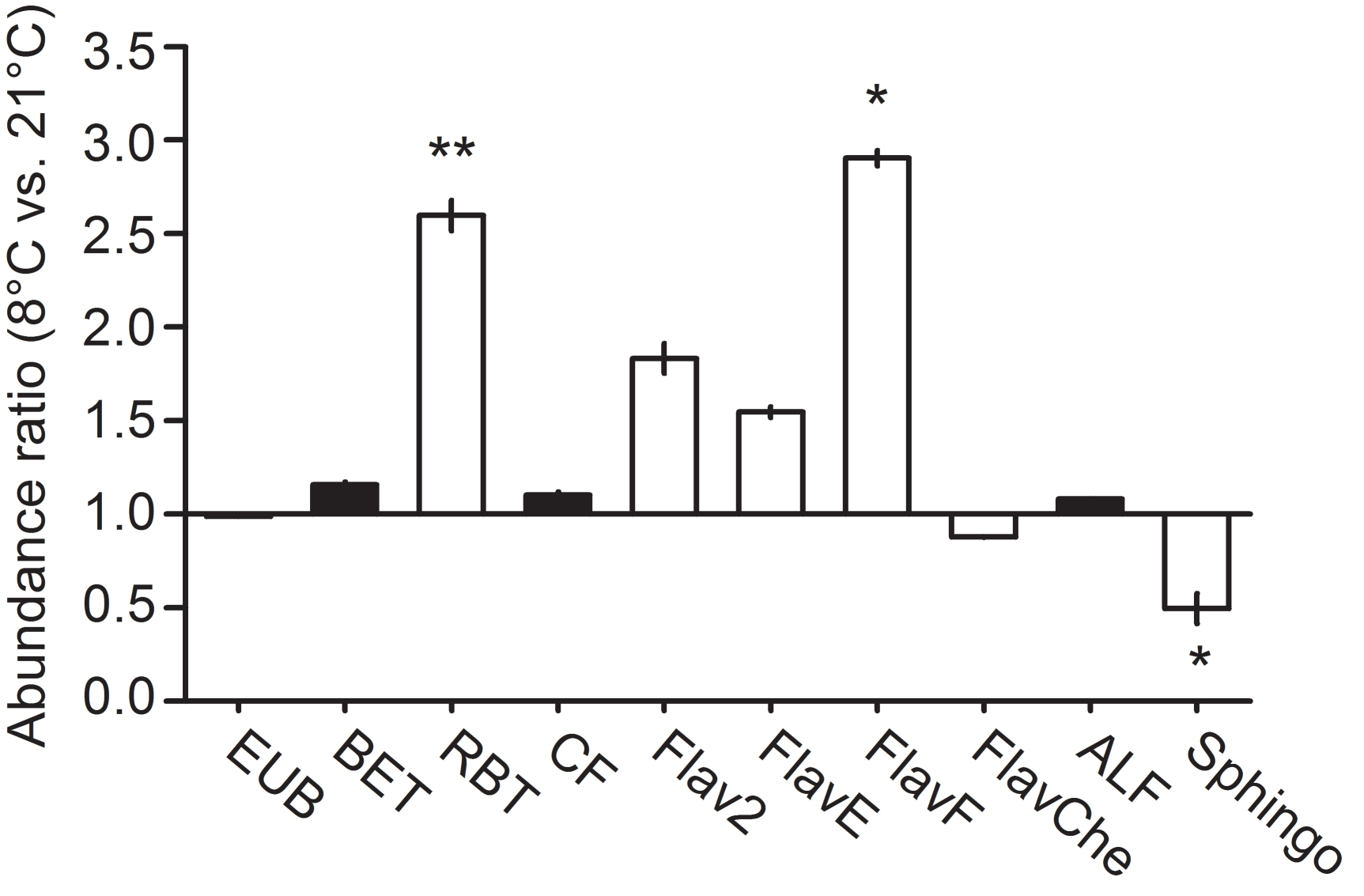

Winter
SpB
CW
SuB
Autumn
Chl a (µg l-1), Temp (°C)
25
20
15
10
5
0
J
F
M
A
M
J
J
A
S
O
N
D
P. rubescens
Diatoms
Chlorophytes
Cryptophytes
Temperature
Experiments

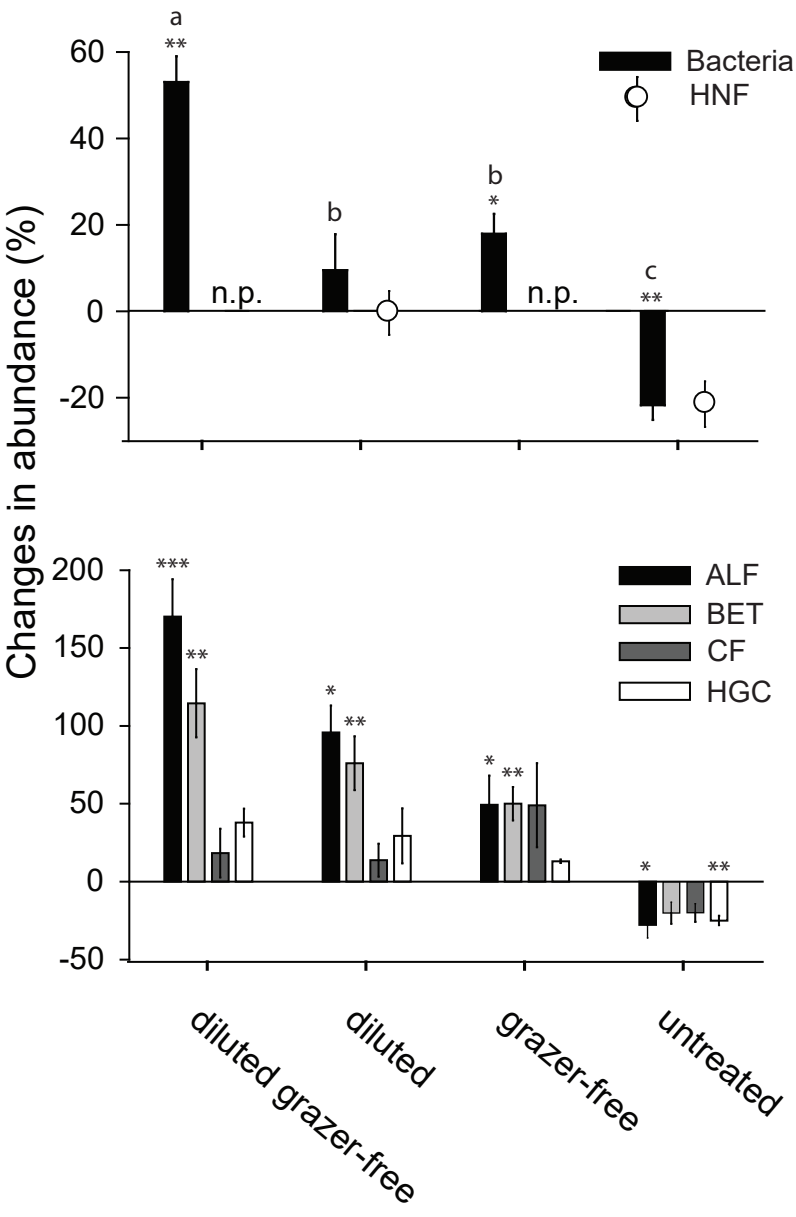

original
enriched
Abundance (10^6 cells ml^{-1})
1.0
0.8
0.6
0.4
0.2
0.0
HNA cells (% of DAPI)
80
60
40
20
0
Glucose uptake (% of DAPI)
50
40
30
20
10
0
n.d.
Leucine uptake (% of DAPI)
50
40
30
20
10
0
n.d.
Feb 02
Apr 03
Apr 23
May 27
Jul 27
Sep 28


original
enriched
HGC
BET
CF
ALF
GAM
growth rate
enrichment factor
Proportion of total cells (%DAPI)
Growth rate (Day-1)
Enrichment factor (final numbers / initial numbers)
b.d.
b.d.
Feb 02
Apr 03
Apr 23
May 27
Jul 27
Sep 28


85%
7/11 summer
97%
13 freshwater
92%
3 /11 spring
FlavB
99%
4/4 summer
FlavE
AB078060, Flavobacterium psychrophilum
74%
spring 7/8
AB455261, Flavobacterium limicola
DQ515962, Flavobacterium glaciei
92%
6/13 summer
FlavF
67%
13/16 spring
FlavC
100%
6/9 summer
68%
EF520552, uncultured clone, Lake Adirondack
78%
GU295971, Flavobacterium sp. ARSA−103, Cheonho reservoir
HE574369, uncultured clone, Lake Zurich
AM230485, Flavobacterium aquatile
100%
63%
7 freshwater
100%
2 marine
75%
3/3 summer
EF407880, Flavobacterium cheniae
FlavCHE
95%
4/4 summer
95%
1/12 summer
90%
18 Tanggulha Lake
83%
DQ372981, Flavobacterium filum
71%
4 freshwater
100%
94%
Fluviicola
100%
Chlorobium
0.01


Original
Enriched
FlavE
FlavF
FlavCHE
Flav2
FlavB
FlavC
CF unidentified
Proportion of CF of total cells (%DAPI)
60
40
20
10
5
0
Feb 04
Apr 03
Apr 23
Mai 27
Jul 27
Sept 28


Abundance ratio (8°C vs. 21°C)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
*
*
EUB
BET
RBT
CF
Flav2
FlavE
FlavF
FlavChe
ALF
Sphingo


Changes in abundance (%)
60
40
20
0
-20
a
**
b
b
*
c
**
n.p.
n.p.
Bacteria
HNF
200
150
100
50
0
-50
***
**
*
**
*
**
*
**
ALF
BET
CF
HGC
diluted grazer-free
diluted
grazer-free
untreated

Supporting information

Table S1. Details of species- to family-like populations in the dilution experiments. Experiments conducted during algal blooms are marked in grey.

Date	Population	% of DAPI		% of CF, BET, ALF		Growth rate (d^{-1})	Enrichment factor
		beginning	end	beginning	end		
Feb 04	Flav2	1.2 ± 0.1	19.9 ± 2.2	31.9	83.9	0.42	38.8
	FlavF	<0.14	3.9 ± 2.6	<3.8	16.5	>0.43	>36.4
	R-BT	4.0 ± 0.2	19.8 ± 1.4	39.2	86.6	0.27	9.9
	Sphingo	0.7 ± 0.2	1.4 ± 0.4	12.1	12.0	0.16	4.7
Apr 03	Flav2	0.2 ± 0.0	8.3 ± 0.3	4.6	32.4	0.66	102.9
	FlavF	<0.14	5.1 ± 0.4	<2.9	20.2	>0.65	>94.5
	R-BT	5.6 ± 0.2	27.4 ± 1.5	44.8	55.8	0.36	12.8
	Sphingo	0.5 ± 0.3	4.0 ± 0.1	13.0	38.5	0.46	35.5
Apr 23	Flav2	0.7 ± 0.1	2.9 ± 0.3	8.0	4.7	0.74	9.4
	FlavB	0.6 ± 0.1	10.4 ± 0.3	6.0	16.7	1.26	43.6
	FlavC	2.6 ± 0.1	37.8 ± 3.9	28.0	60.2	1.16	33.5
	FlavF	<0.14	4.5 ± 0.4	<1.5	7.2	>1.43	>73.4
	R-BT	6.5 ± 0.3	2.6 ± 0.2	44.7	55.8	-0.03	0.9
	Sphingo	1.3 ± 0.2	5.5 ± 0.4	41.6	103.1	0.77	10.5
May 27	Flav2	0.6 ± 0.1	1.5 ± 0.3	9.1	18.9	0.31	4.4
	FlavF	<0.14	1.3 ± 0.1	<2.2	16.6	>0.94	>16.8
	R-BT	1.0 ± 0.3	5.5 ± 0.3	30.1	28.3	0.71	8.7
	Sphingo	0.5 ± 0.1	4.4 ± 0.3	53.9	73.9	0.90	15.2
Jul 27 22°C	Flav2	1.3 ± 0.0	7.9 ± 0.2	38.5	17.3	1.99	19.8
	FlavE	0.4 ± 0.0	2.6 ± 0.2	10.6	5.8	2.05	22.1
	FlavF	<0.14	3.6 ± 0.3	<3.6	7.8	>2.96	>84.6
	FlavCHE	0.6 ± 0.1	24.5 ± 0.7	16.2	53.8	3.26	133.2
	R-BT	2.2 ± 0.4	1.3 ± 0.2	21.1	25.3	0.45	2.0
	Sphingo	1.4 ± 0.1	16.6 ± 1.2	39.0	63.9	2.43	38.7
Jul 27 8°C	Flav2	1.2 ± 0.1	14.4 ± 2.4	35.8	28.4	0.51	35.6
	FlavE	0.3 ± 0.0	4.1 ± 0.2	6.5	8.3	0.54	46.4
	FlavF	<0.14	10.4 ± 0.7	<3.5	20.8	>0.76	>201.5
	FlavCHE	0.6 ± 0.0	21.5 ± 0.7	14.7	44.1	0.66	103.3
	R-BT	2.4 ± 0.1	3.3 ± 0.2	15.7	59.7	0.20	4.1
	Sphingo	1.9 ± 0.3	8.3 ± 1.8	52.2	29.5	0.35	12.2
Sep 28	R-BT	2.0 ± 0.3	1.6 ± 0.3	34.3	28.4	0.04	1.2
	Sphingo	0.3 ± 0.0	4.9 ± 1.1	12.9	47.0	0.76	22.6

% of CF, BET, ALF: contribution of species-like populations Flav2, FlavB, FlavC, FlavE, FlavF, and FlavCHE to all *Cytophaga-Flavobacteria* (CF), of *Limnohabitans* spp. (R-BT) to all *Betaproteobacteria* (BET), and of *Sphingobacteriaceae* (Sphingo) to all *Alphaproteobacteria* (ALF), respectively. Enrichment factor: Ratio of a population's abundance at the end vs. at the beginning of an experiment.

Figure S1. Examples of cytograms of dilution experiments (May 27) before (top) and after (bottom) an incubation period of 72 h. HNA, cells with high nucleic acid contents; LNA, cells with low nucleic acid contents. The lowest population was excluded from the analysis since the majority of the signals are not associated with bacteria. Earlier results showed that on average 3.5% of those particles can be retained on 0.2 µm membrane filters and hybridized with a general probe for bacteria (EUB I-III; R. Freimann, unpublished).